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9404575 PREDICTING THE SPREAD OF INSECT RESISTANCE: A TOOL TO EVALUATE ECOLOGICAL RISK

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FINAL REPORT:

Wild plant species produce plant defenses that the biotechnology industry hopes to exploit. In particular, crop plants are being developed that can express high levels of toxins that normally are found at lower levels in natural plant communities. These transgenic crops are likely to favor the evolution of "tolerance" or "resistance" among pests that specialize on the engineered crops. Evolution on the part of pests to toxins has happened repeatedly, and there is little doubt that pests will similarly evolve resistance to these natural plant toxins. However, pests that are resistant to natural plant toxins pose a much greater threat to natural plant communities than pests that are resistant to other insecticides.

There were three specific aims of the proposed research:

1. To minimize the threat of the evolution of natural toxin resistance in pests, we developed a predictive evolutionary model to explore the consequences of various management tactics.
We then sought to test model predictions with experiments in which diamondback moths were subjected to rapeseed plants into which genes for a proteinase inhibitor and/or a Bt delta-endotoxin had been introduced. In these experiments, we tracked the rate at which resistance evolves for a variety of resistance management tactics.
3. Since the risk of the evolution of natural toxin resistance may translate to an ecological risk to natural plant systems that carry these toxins, we planned to explore the fate of resistant pests on wild plant relatives of *Brassica napus* that contain proteinase inhibitors.

Results

We began our work by creating transgenic *Brassica napus* plants expressing either the Bt deltaendotoxin or the potato proteinase inhibitor II (abbreviated PI). These lines were then crossed to create plants that expressed Bt, PI, both PI/Bt or neither. Lines were created to examine how the distribution of these two plant toxins impacts that rate at which resistance spreads through a population of diamondback moths *(Plutella xylostella)* and the extent to which plants are damaged by the evolving pest population. We were particularly interested in comparing the dynamics of diamondback moths when subjected to plant populations composed of (l) individual plants expressing both toxins, (2) a spatial mixture of plants that each express a single toxin, and (3) plants expressing single toxins that are rotated each generation. Certain changes in the proposed research plan were required after learning that the proteinase inhibitors actually lead to an increase, rather than decrease, in damage caused by diamondback moths (for details, see section #2 below). In particular, the transgenic plant lines that we had created (+/- PI, +/Bt, +/- both PI and Bt) did not allow us to investigate strategies for the deployment of two plant toxins. Instead, we examined how refuges provided by PI or by the absence of both PI and Bt influenced evolutionary dynamics. We were furthermore unable to ask how resistance to a natural toxin impacted ecological interactions (goal #3 above). Instead, we examined in more detail the ecology of PI resistance in *Brassica napus.*

1. Models of the evolution of resistance

We used a one-locus, two-allele, haploid population genetic model to describe the evolution of insect resistance to a single toxin. Our model makes several assumptions that are common to population genetic models: (1) the insect population is large and constant, (2) the generations of the insect are discrete, (3) there is a single resistance locus, (4) there are no pleiotropic fitness costs of insect resistance, and (5) insects choose their host plants and their mates at random. We used this model to track the growth of insect populations to each of three resistance management strategies - spatial mosaics, temporal mosaics, and pyramiding of the two plant toxins. In our model exploration, two separate indices were considered. First, we considered the widely used index, "time-to-resistance". Previously published work has been shown that strong selection leads to fast evolution of insect resistance; any resistance strategy that lowers the apparent strength of selection therefore prolongs the time to resistance. As a result, time-to-resistance is increased by weak toxins and by the incorporation of refuges into plant populations. A key assumption in these arguments is that insects below the critical fitness threshold have no detrimental effect on toxic plants. It is more likely, however, that the ability of insects to damage plants is a continuous function of insect fitness. To incorporate this biology, we developed a second index called "insect-fitness-suppression" which is a measure of both the time-to-resistance and the total damage inflicted on plants during that time. A successful strategy is one that maximizes insect-fitness-suppression. There are several conclusions, of relevance to the management of resistance, that follow from the investigation of our models. A few of the most important, key results are summarized below:

· Time-to-resistance and insect-fitness-suppression make different predictions about the merits of weak toxins and refuges. Whereas the time-to-resistance model predicts that relatively weak toxins and refuges are always advantageous, the insect-fitness-suppression model predicts that intermediate levels of plant toxins are superior. Thus, it is important to distinguish which better reflects the biology underlying plant-pest interactions.

· Time-to-resistance models assume that pests inflict no damage on plants prior to the evolution of resistance, whereas insect-fitness-suppression models assume a linear relationship between insect fitness and damage. Experimental investigations indicate that the assumptions of the insect-fitness suppression model are more accurate for our system.

. The insect-fitness-suppression model predicts that rather than being universally useful, refuges rarely improve insect fitness suppression. Refuges are only beneficial when they are provided in the presence of very strong toxins, and they are distributed in space but not in time.

These results are important in redirecting our attention on insect-fitness-suppression rather than time-to-resistance models, and in debunking the widespread belief that refuges are always beneficial in terms of resistance management. Our insect-fitness-suppression model will be applicable to many systems and could change the way we think about the management of pest resistance.

2. **Experimental tests of the role of refuges in resistance management**

We performed two large experiments in controlled environment rooms. In the first, we compared the effect of the distribution of two transgenes (Bt delta-endotoxin and PI-II), both *in Brassica napus* var. Westar, on the rate of evolution of resistance in diamondback moths *(Plutella xylostella).* Specifically, we compared how moths evolved on plants with neither transgene (null treatment), both transgenes in every plant (constant exposure to Bt), or spatial mixtures of the transgenes (spatial refuge). We measured the evolution of resistance in two ways: as an increase in larval survival rates on transgenic plants, and as an increase in moth population growth rates.

We found that diamondback moths evolve in controlled environmental conditions. Bt has a strong pesticidal effect on susceptible moths (0.76 decrease in survival and 0.91 decrease in population growth relative to nulls). After four generations, some moth populations evolved much higher population growth rates on Bt (as high as for moths on null plants). We saw no evolution of moths on plants with PI-II. Evolution was faster on homogeneous Bt treatments, but in these treatments moths did a poor job of surviving in early generations. The early advantage of homogeneity was not sufficient to outweigh the advantage of very slow evolution of moths with spatial refuges. Thus, our experiments showed that for preservation of susceptible moths and the long-term use of Bt, spatial mosaics were more effective than homogeneous distribution of the toxin.

In the second experiment, we evaluated whether there was a non-additive interaction effect of the two transgenes on the evolution of insect resistance by exploring the hypotheses that diamondback moth fitness was unaffected by serine protease inhibitors, and that protease inhibitors did not alter the ability of *Bt* to protect plants from diamondback moths. Prior study of the effect of protease inhibitors on diamondback moths suggests that moths are resistant to them, and thus protease inhibitors represent an ineffective defense against moths. Our data suggest instead that diamondback moths *do* suffer lower growth rates when they consume plants with foreign protease inhibitors, but that effect is hidden by increased compensatory consumption. Plants, instead of gaining an advantage by lowering the insect growth rate, suffer a disadvantage as moths consume more tissue to mitigate their effect. Furthermore, PI used in conjunction with another transgenic pesticidal protein, *Bt,* makes *Bt* less effective at protecting plant tissue. Thus, transgenic proteinase inhibitors are not only useless in protecting *Brassica* plants from diamondback moths, they are actually destructive.

PUBLICATIONS CITING SUPPORT OF THIS AWARD

Bergelson, J., Winterer, J. and C. B. Purrington. 1998. Ecological impacts of transgenic crops. For: *Biotechnology and Genetic Engineering of Plants (V.* Malik, ed). Oxford Universit~v Press. In press.

Wintererer, J. and J. Bergelson. Diamondback moths compensate for the presence of protease inhibitors by eating more. In Preparation for J. Economic Entomology.

Winterer, J. and J. Bergelson. The role of refuges in the evolution of insect resistance to a plant toxin. In Preparation for Ecological Applications.

Winterer, J. Maintenance of variation in a heritable plant defense? Trichome density in *Brassica.* In Preparation for Oecologia.

PRESENTATIONS AT NATIONAL MEETINGS (describing research funded by this award)

Experiments to test the predictive value of a model that suggests how to manage transgenic crops. USDA Risk Assessment Conference. Ottawa, Canada.

Effective use of transgenic crops to manage evolving pathogens. Annual meeting of Society for the Study of Evolution. McGill University, Montreal, Canada.

Predicting the spread of insect resistance: a tool to evaluate ecological risk. USDA Risk Assessment Conference. Pensacola Beach, Florida, USA

The role of refuges in the evolution of insect resistance to plant defenses. Annual meeting of the Ecological Society of America, Baltimore, MD, USA